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INDUCTION OF THE WRINKLED BLASTULA FORMATION IN THE STARFISH, *ASTERINA PECTINIFERA*, BY MODIFIED DEVELOPMENTAL CONDITIONS

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The wrinkled blastula stage has been designated as a normal and definite stage in the embryonic development of a number of species belonging to the two classes of Echinodermata, Asteroidea and Holothuroidea (Newth, 1925; Dan, 1957; Hayashi, 1972). In Asteroidea more than ten species, such as *Solaster endeca* (Gemmill, 1912, 1916), *Porania pulvillus* (Gemmill, 1915), *Astropecten aranciacus* (Hörstadius, 1939), *Leptasterias hexactis* (Chia, 1966, 1968), *Mediaster aequalis* (Birkeland, Chia and Strathmann, 1971), *Certonardoa semiregularis* (Hayashi and Komatsu, 1971) and *Astropecten latespinosus* (Komatsu, 1975), have hitherto been reported as the species which pass through the wrinkled blastula stage in their normal course of development. Hayashi (1972) classified various breeding habits and larval forms of asteroids, with remarks on the wrinkled blastula, into six groups, and he suggested that the occurrence of the wrinkled blastula in some species might be due to the accumulation of a large amount of yolk during oogenesis in those species, although a close phylogenic relationship could not be detected between the species with the wrinkled blastula stage and between the species without the stage.

In the starfish, *Asterina pectinifera*, the most common species on the Japanese coast, Dan (1957) described briefly that no such phenomenon as forming a morula with many surface foldings and wrinkles could be observed. Komatsu (1972) observed the wrinkled blastula stage of this species collected on the coast of Toyama Bay, Sea of Japan, and she pointed out that the wrinkled blastula stage was inevitable in the normal course of development of this species. The recent report of Dan-Sohkawa and Satoh (1978), however, showed that most of the embryos of this species collected on the coast of Tokyo Bay, Pacific Ocean, did not form wrinkled blastulae. These seemingly contradictory reports indicate at least two possibilities on the wrinkled blastula formation of this species. One is the possibility that the wrinkled blastula formation is a rigidly planned process within the batches in some localities, and the occurrence of a wrinkled blastula would not be changed by modified environmental conditions. The alternative is that the wrinkled blastula formation of this species is rather flexible, and the formation could be modified if developmental conditions were manipulated.

The present study examines these alternative possibilities. At first, the occurrence of wrinkled blastula on two different coasts was observed in natural sea water. The results indicated that the occurrence of wrinkled blastula in this species differed from 10 to 90% among batches. Then, effects of several agents or conditions, such as osmotic pressure, salinity, temperature, pH and Ca^{++}, Mg^{++}, SO_4^{--} concentration of sea water, on the ratio of appearance of wrinkled blastula

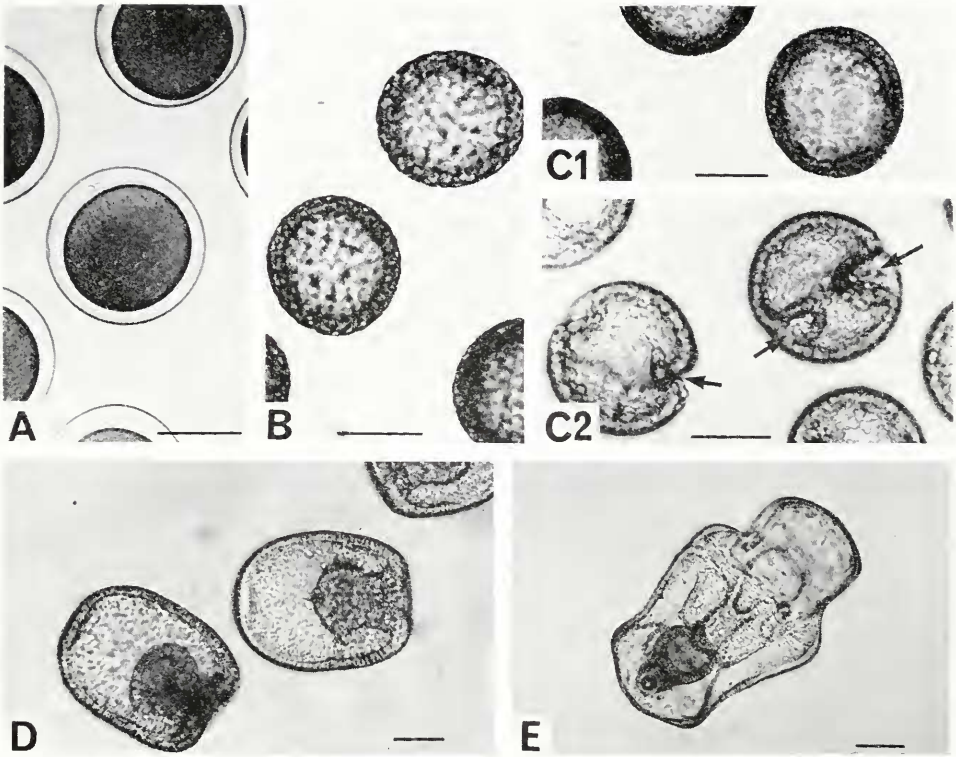

FIGURE 1. Normal embryonic development of the starfish, *Asterina pectinifera:* A, fertilized egg, 1.5 hours after insemination; B, early blastula, 5 hours after insemination; C, middle blastula, 7 hours after insemination [(1) nonwrinkled blastula with smooth surface and (2) wrinkled blastula with egression tracts (arrow)]; D, gastrula, 20 hours after insemination; and E, bipinnaria, 3 days after insemination. Mouth, oesophagus, round stomach and intestine are visible. Bar equals 100 μm.

were examined. It became clear that the wrinkled blastula formation could be increased remarkably by allowing the eggs to develop in sea water of low osmotic pressure.

MATERIALS AND METHODS

Adults of the starfish *Asterina pectinifera,* were collected on the coast of Tateyama Bay (a part of Tokyo Bay), Pacific Ocean, in June of 1977 and on the coast of Mutsu Bay, Tsugaru Channel, in September of 1977 during the presumed breeding season. By treatment with 1-methyladenine of the ovaries which were removed from the females according to Kanatani (1969), fertilizable ova were obtained. It has been shown that the wrinkled blastula formation of this species is not an artifact caused by the treatment of the eggs with 1-methyladenine (Komatsu, 1972). Dilute sperm suspension was prepared just before the insemination from mature testes removed from the males and added to the dish containing the ova.

Usually about a hundred eggs were reared in a Petri dish containing the various types of test solutions from just after the insemination. Experiments were carried out at laboratory temperature (20–22° C).

Filtered natural sea water, artificial sea water (Jamarin U, Jamarin Laboratory Co., Osaka, Japan) and Herbst's artificial sea water were used in this study. Contents of major elements in normal strength Jamarin U were as follows (g/liter): Cl = 16.947, Na = 9.311, Mg = 1.137, S = 0.774, Ca = 0.395, and K = 0.344, and its osmolarity was 0.874 osmole. Formulas for Herbst's was as follows (g/liter): NaCl = 26.3, KCl = 0.7, $MgSO_47H_2O$ = 11.9, $CaCl_22H_2O$ = 1.5, and $NaHCO_3$ = 0.5, and its osmolarity was about 1.030 osmole (Hosokawa and Oshima, 1971).

Results

The eggs were translucent, about 170 μm in diameter (Fig. 1A). At laboratory temperature (20–22° C), they repeated cleavages every 30–40 minutes and developed into hollow blastulae about 5 hours after insemination (Fig. 1B). When the embryos developed into the wrinkled blastulae, streaks (egression tracts) appeared on the surface of the blastulae about 5.5 hours after insemination (Fig. 1C). The egression tracts gradually increased in number and size, and embryos reached the most wrinkled stage about 7 hours after insemination. Therefore, the ratio of wrinkled blastula formation was examined about 7 hours after insemination in 100–150 randomly selected eggs of each batch. The distinction between wrinkled and nonwrinkled blastulae was clear enough to exclude the possibility of misjudgement (Fig. 1C).

Five hours after the beginning of wrinkling, the surface of the blastulae resumed smoothness by the fusion of neighboring egression tracts. Eleven hours after insemination, the embryos hatched out and 30 minutes thereafter true gastrulation took place (Fig. 1D). They developed into typical bipinnaria larvae about 3 days after insemination (Fig. 1E) . Since the embryos which passed through the wrinkled blastula stage developed into typical bipinnaria larvae, the wrinkled blastula stage seems to be a completely normal process, as was reported earlier (Komatsu, 1972).

Occurrence of wrinkled blastula in natural sea water

In nine batches of eggs from Tateyama Bay, the occurrences of wrinkled blastula in the natural sea water of Tateyama Bay were 12, 14, 16, 19, 23, 40, 53, 53, and 69%, respectively. The occurrences of wrinkled blastula of ten batches from Mutsu Bay were 8, 12, 16, 17, 25, 26, 39, 41, 83, and 95%, respectively, when the eggs developed in the natural sea water of Mutsu Bay. This result indicated that the eggs of *Asterina pectinifera* pass through the wrinkled blastula stage, although the formation ratios were rather different among batches and the ratio was very low (10–25%) in more than half of batches examined.

Effects of osmotic pressure and salinity of sea water

The occurrences of wrinkled blastula in nine batches from Tateyama Bay, when the eggs developed in their natural sea water diluted with various amounts of dis-

tilled water, were examined. As clearly shown in Figure 2, the occurrences of wrinkled blastula increased in every batch in proportion to decrease in the concentration of natural sea water. For example, the batch shown by open circles, which developed about 10% wrinkled blastulae in natural sea water of normal concentration, formed more than 95% wrinkled blastulae in 0.8 times diluted sea water.

In order to examine the wrinkled blastula formation ratio in both diluted and concentrated sea water, artificial sea water, Jamarin U, was used. The result of ten batches from Tateyama Bay is shown in Figure 3. Similar to the case of the natural sea water, the occurrence of wrinkled blastula increased conspicuously in proportion to decrease in concentration of Jamarin U. In the concentrated Jamarin U (1.1–1.2 times), however, the occurrence of wrinkled blastula neither increased nor decreased. The eggs developed abnormally in 1.3 times concentrated Jamarin U.

Nearly all eggs developed into wrinkled blastulae in 0.8 times diluted natural sea water (Fig. 2) and in 0.9 times diluted Jamarin U (Fig. 3). The osmolarity of natural sea water of Tateyama Bay was about 0.980–1.020 osmole (Hosokawa and Oshima, 1971), while that of Jamarin U of normal strength was 0.874 osmole.

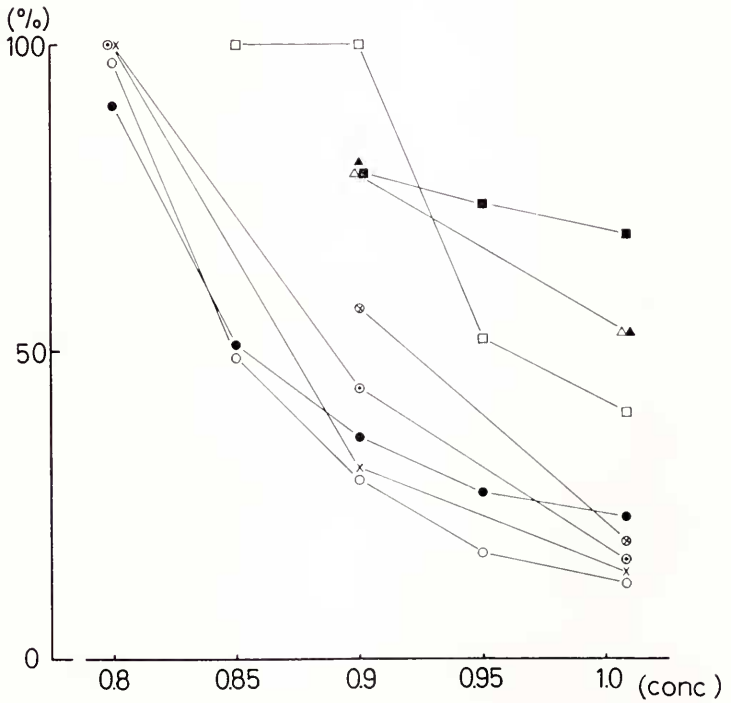

FIGURE 2. The percentage of wrinkled blastula formation in nine batches from Tateyama Bay in various concentrations of its natural sea water: the abscissa shows the ratio of wrinkled blastula formation (%); the ordinate, relative value of concentration of natural sea water diluted with distilled water (1.0 shows the normal concentration of natural sea water). The same marks tied with lines show the eggs of the same batch.

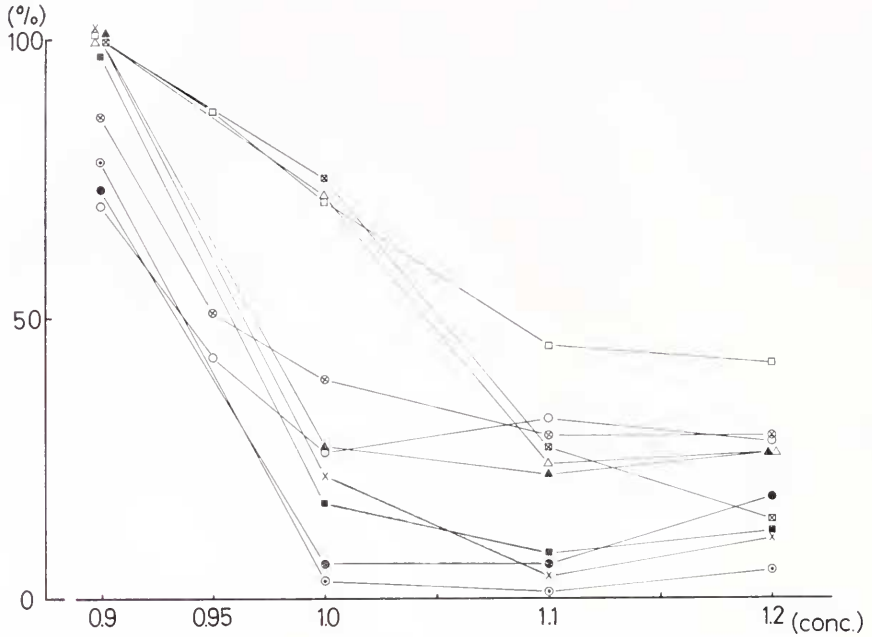

FIGURE 3. The percentage of wrinkled blastula formation in ten batches from Tateyama Bay in various concentrations of artificial sea water, Jamarin U. The same marks tied with lines show the eggs of the same batch.

Therefore, the osmolarity of 0.8 times diluted natural sea water almost corresponded to that of 0.9 times diluted Jamarin U.

The effect of dilution on the wrinkled blastula formation ratio was ascertained using Herbst's artificial sea water. The occurrences of wrinkled blastula in three batches from Mutsu Bay in Herbst's diluted with various amounts of distilled water are shown in Figure 4. The occurrence of wrinkled blastula of every batch increased in proportion to the decrease in concentration of Herbst's (Fig. 5A, B).

In order to discover whether the effect of low concentration of sea water might be due to modified osmotic pressure or due to modified salinity, the osmotic pressure of diluted sea water was maintained at normal strength by diluting the sea water with 0.75 M sucrose solution, which has been shown to be of almost the same osmotic pressure with the normal Herbst's (Hosokawa and Oshima, 1971), instead of distilled water. In all three batches, the occurrences did not increase in proportion to decrease of the saline concentration of Herbst's diluted with 0.75 M sucrose solution (Fig. 4 and 5C).

In every case the embryos which passed through the induced wrinkled blastula stage developed into normal gastrulae (Fig. 5E), although the egression tracts remained until the early gastrula stage in some cases. They became normal bipinnaria larvae.

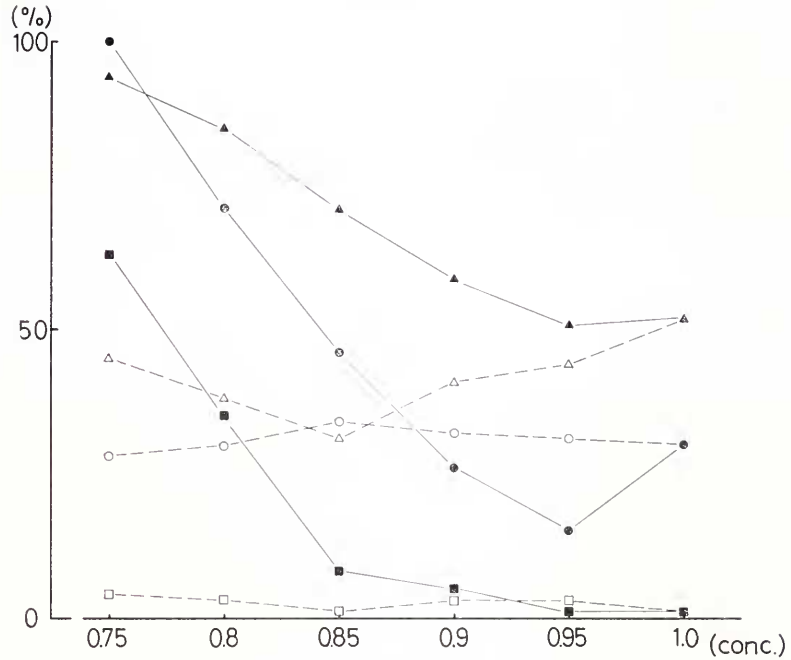

FIGURE 4. The percentage of wrinkled blastula formation in three batches from Mutsu Bay in various concentrations of Herbst's artificial sea water. The same marks, circles, triangles and squares, show the eggs of the same batch. Open marks show the ratios of wrinkled blastula formation when the Herbst's was diluted with 0.75 M sucrose solution to maintain the same osmotic pressure of the solution of different salinity, and solid marks show the ratios of wrinkled blastula formulation when the Herbst's was diluted with distilled water.

Effects of temperature

The occurrences of wrinkled blastula in two batches from Tateyama Bay and five batches from Mutsu Bay were examined at various developmental temperatures. In this experiment, all embryos were reared in Jamarin U or Herbst's of normal concentration. The occurrence of wrinkled blastula at low temperatures (13–15° C) were examined about 12 hours after insemination because of the delay of development (Fig. 6). Although the wrinkled blastula formation ratio of two batches slightly decreased at low temperatures, there was no conspicuous change in the ratio at 13–23° C. In every batch the occurrence of wrinkled blastula increased at high temperatures (25–26° C), but at such high temperatures most embryos became abnormal.

Effects of pH

The pH of normal Herbst's was adjusted by adding 30 mM tris-amino methane —0.1 N HCl, and the pH of used solution was examined again after the experiment. The occurrences of wrinkled blastula in six series of three batches from Mutsu Bay in sea water of various pH values are shown in Figure 7. Between the range

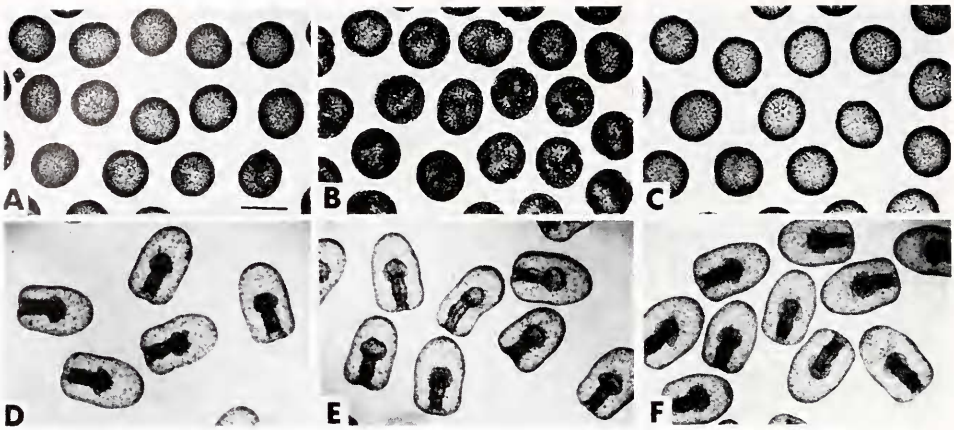

FIGURE 5. Induced wrinkled blastula formation of the eggs developed in Herbst's of low osmotic pressure. A, B, and C show the blastulae 8 hours after insemination: A, normal Herbst's; B, 0.75 times diluted Herbst's with distilled water; C, 0.75 times diluted Herbst's with 0.75 M sucrose solution. D, E, and F show the late gastrulae 24 hours after insemination: D, the late gastrulae developed from eggs shown in A; E, those from eggs shown in B; F, those from eggs shown in C. All eggs in the photographs are of one batch from Mutsu Bay. Bar equals 200 μm.

of pH 7.2–8.7, appreciable changes of the ratios were not detected. All eggs developed normally in this range of pH.

Effects of Ca^{++}, Mg^{++} and SO_4^{--} concentration

Among inorganic components of Herbst's, Ca^{++}, Mg^{++}, and SO_4^{--} were examined for their effects, since Ca^{++} and Mg^{++} have been shown to be involved in cell-to-cell adhesion, while SO_4^{--} plays some role in morphogenesis. The concentration of Ca^{++}, Mg^{++} and SO_4^{--} was changed, respectively. In this experiment Ca^{++} or Mg^{++} was exchanged for Na^{+}, and SO_4^{--} for Cl^{-}, so the total valency changed very little between the test solutions. A batch with a high wrinkled blastula formation ratio and that with a low ratio from Mutsu Bay were used. The results are summarized in Figure 8. Between the range of concentrations examined, the occurrence of wrinkled blastula did not show appreciable increase or decrease. A batch in Ca^{++}-free sea water and a batch in 1.8 times SO_4^{--} concentrated sea water increased considerably the ratio of wrinkled blastula, but the former eggs developed abnormally.

DISCUSSION

The present study suggests two results: first, the occurrence of wrinkled blastula in *Asterina pectinifera* is considerably different among batches collected both from Tateyama Bay and Mutsu Bay; and secondly, the wrinkled blastula formation can be induced by allowing the eggs to develop in sea water of low osmotic pressure.

Most reports on the wrinkled blastula in asteroids, hitherto, indicated that the

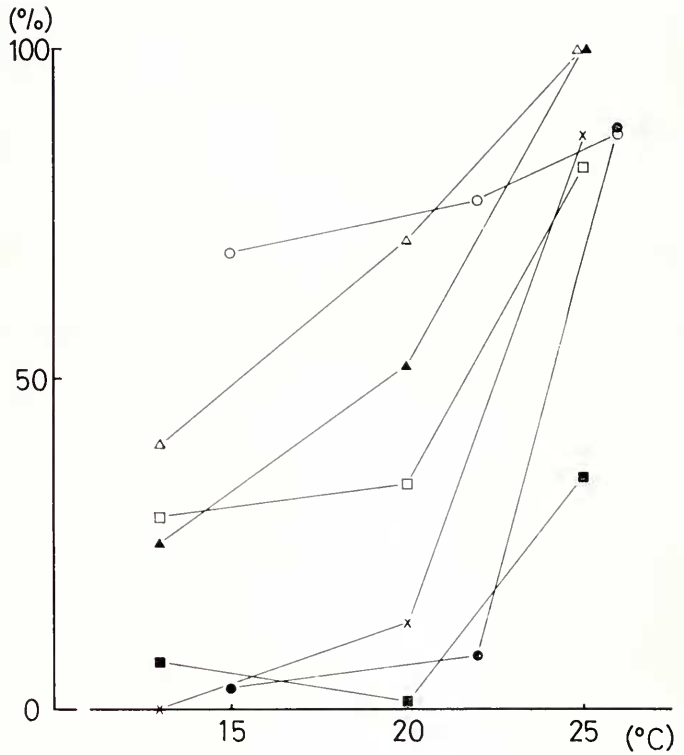

FIGURE 6. The percentage of wrinkled blastula formation in two batches from Tateyama Bay (shown by the open and solid circles) and five batches from Mutsu Bay developed at various temperatures. The same marks show the eggs of the same batch.

eggs of a certain species pass through the wrinkled blastula stage, while other species do not form the wrinkled blastula (reviewed by Hayashi, 1972); that is, occurrence of wrinkled blastula seems to have been regarded as a feature characteristic of species.

In *Asterina pectinifera,* Komatsu (1972) reported that the eggs obtained from Toyama Bay, Sea of Japan, inevitably pass through the wrinkled blastula stage. On the other hand, Dan (1957) and Dan-Sohkawa and Satoh (1978) reported that most of the embryos of this species collected on the coast of Tokyo Bay, Pacific Ocean, did not form wrinkled blastulae. However, these reports did not present any actual percentage of wrinkled blastula formation. The present results indicate that the occurrence of wrinkled blastula was very low (10–25%) in more than half of the batches examined. Since many eggs of the species from both Tateyama Bay and Mutsu Bay developed, without passing through the wrinkled blastula stage, into normal bipinnaria larvae, the wrinkled blastula stage is not an integral part of this starfish's development. In the European species, *Asterina gibbosa,* Newth (1925) noted the occurrence of a wrinkled blastula stage, although MacBride (1896) did not mention wrinkling in the same species. It is worth

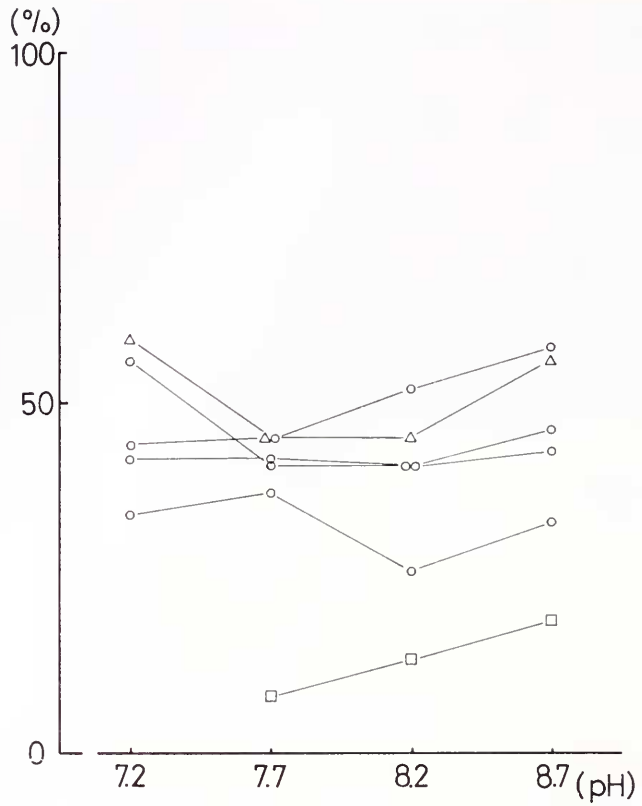

FIGURE 7. The percentage of wrinkled blastula formation in six series experiments of three batches from Mutsu Bay in sea water of various pH values. The same marks show the eggs of the same batch.

mentioning the possibility that in *A. gibbosa,* some eggs pass through the wrinkled blastula stage while others do not, similar to the case of *A. pectinifera* which has been clarified in the present study. These species are of special significance for analyzing the nature of the wrinkled blastula.

This study seems to be the first experimental analysis of the phenomenon of the starfish's wrinkled blastula. The results of the present study show clearly that sea water of low osmotic pressure increases the wrinkled blastula formation ratio, but modified salinity, temperature, pH, and Ca^{++}, Mg^{++}, SO_4^{--} concentrations give no effect on wrinkled blastula formation. Whether or not such low osmotic sea water pressures would happen naturally is not certain. The induced wrinkled blastula continued to develop and became normal bipinnaria larva in such low osmotic sea water. The mechanism involved in the induction of wrinkled blastula formation, as well as the wrinkled blastula formation in normal developmental conditions, is still obscure. In any event, much more information is needed for an understanding of the wrinkled blastula of starfish.

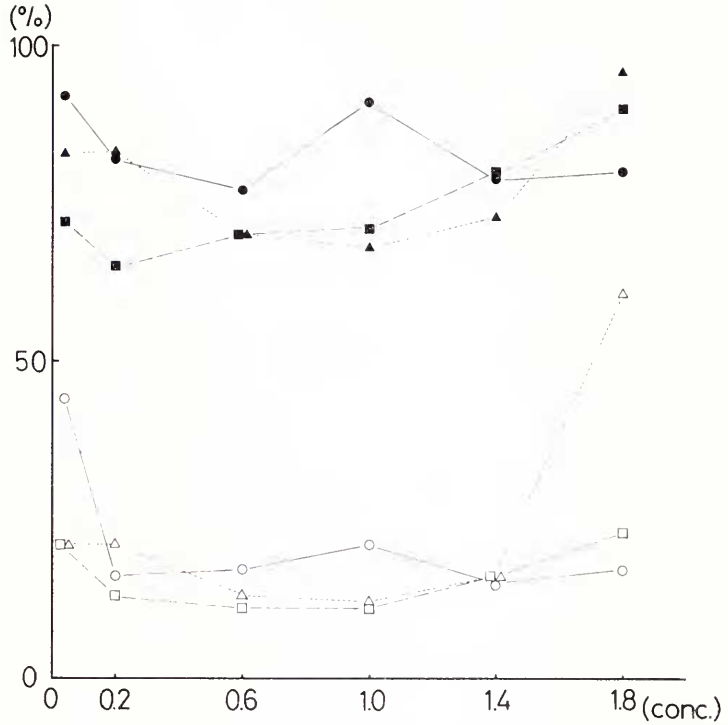

FIGURE 8. The percentage of wrinkled blastula formation in two batches from Mutsu Bay developed in sea water of various Ca^{++}, Mg^{++}, and SO_4^{--} concentrations. Ordinate shows the relative value of each ion concentration (1.0 shows the normal concentration of each ion in Herbst's artificial sea water). Circles show the ratios in various concentrations of Ca^{++}; squares, Mg^{++}; triangles, SO_4^{--}. Open marks show the first batch; and solid marks, the second.

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SUMMARY

1. The occurrence of the wrinkled blastula in the starfish, *Asterina pectinifera*, was examined. The ratios were different, from 10 to 90%, among batches. The wrinkled blastula stage is not always an inevitable process during the embryonic development of this species.

2. Low osmotic sea water (both natural and artificial sea water) remarkably increased the ratio of wrinkled blastula, while modified salinity, temperature, pH and Ca^{++}, Mg^{++}, SO_4^{--} concentrations of sea water gave no effect on the ratio. The induced wrinkled blastula developed into normal bipinnaria larva.

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